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REVIEW

Toward 'SMART' stem cells

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Stem cell research is at the heart of regenerative medicine, which holds great promise for the treatment of many devastating disorders. However, in addition to hurdles posed by well-publicized ethical issues, this emerging field presents many biological challenges. What is a stem cell? How are embryonic stem cells different from adult stem cells? What are the physiological bases for therapeutically acceptable stem cells? In this editorial review, I will briefly discuss these superficially simple but actually rather complex issues that surround this fascinating cell type. The goal of this special issue on stem cells in Gene Therapy *is to review some fundamental and critical aspects of current stem cell research that have translational potential.*
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The promise of stem cell research

Stem cell research is moving to center stage in the biomedical field. The main driving force for this is the demand for regenerative medicine, which offers hope for the treatment of many devastating degenerative diseases such as diabetes, Parkinson's disease and Alzheimer's disease. The field has already demonstrated what is considered a classic example, that is, hematopoietic stem cell (HSC) transplantation, which has been used in the clinic to cure or treat hematopoietic failures, immune deficiencies, leukemias and certain other cancers. There is great potential for significant health gains in a much broader scope through the use of stem cell therapy in conjunction with other medical modalities. For instance, the first-line treatment of chronic myeloid leukemia (CML)—a well known HSC cancer with small molecule ABL kinase inhibitors has been a great success story of molecular medicine.[1] However, CML remains an incurable disease likely due to the inability of the current inhibitors to eradicate CML-initiating cells or the so-called 'leukemic stem cells'.[2] To cure CML, it will be necessary to target CML-leukemic stem cells[3] specifically.

Stem cell research has been associated with gene therapy since its establishment. It continues to provide a complementary aspect to gene therapy because the self-renewing and differentiative properties of stem cells make them the ideal vehicle for therapeutic transgenes in their progeny.[4] The complete cure of adenosine deaminase-deficient patients by HSC gene therapy represents a remarkable success in the use of combinational stem cell and gene therapies to manage an inherited disorder.[5] A recent study demonstrated that HSCs can carry a specific antigenic protein which, once the HSCs differentiate into T cells, will eradicate a tumor.[6] This suggests a broad application of HSCs therapy in conjunction with immunotherapy and gene therapy for treating a broad spectrum of cancers.

Given the fact that tissue stem cells exist in many organs throughout an organism's lifetime, it seems likely that these cells are involved in the pathogenesis of many diseases, although this cannot be definitively studied until the phenotype of a specific tissue stem cell type is identified. For example, it is known that many types of human leukemia are directly or indirectly transformed at the HSC level.[7,8] Furthermore, the current cancer stem cell or tumor stem cell (TSC) hypothesis postulates that self-renewing cells within a tumor are responsible for tumor growth and renewal.[9,10] Increasing evidence supports the existence of TSCs in solid tumors derived from many organs, including the human breast, brain, colon, pancreas and prostate.[11–16] Stem cell culture toward a specific path or a particular disease state may resemble normal developmental process or pathogenesis, thus offering a new platform for drug discovery and pharmacology/toxicology studies.[17]

For those researchers who are not directly involved in stem cell research, it is worth mentioning that embryonic stem cells (ESCs) have been used as a powerful and indispensable cellular vehicle to carry an exogenous transgene or to target an endogenous gene product in a specific tissue lineage or a differentiated cell type to study the function of a gene at different developmental stages. The discovery concerning the principles for gene targeting in mice via the use of ESCs by Mario Capecchi, Martin Evans and Oliver Smithies has recently won the Nobel Prize in Physiology or Medicine of 2007.

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About the concept of stem cells

Enormous advances in stem cell research have been made, especially since the first human ESC line was established.[18] The stem cell research community is becoming a united scientific entity across basic science, clinical medicine, ethics and bioindustry, yet many concepts in the field remain poorly defined. Even the popular term 'stem cells' has no universally accepted definition.[19–25] This ambiguity has caused confusion regarding what is being studied, especially among the general public.

The term 'stem cells' first appeared in the research literature at least 140 years ago.[26] It was originally used by embryologists to describe germline cells, and by hematologists to describe blood-forming cells.[26] In 1961, Till and McCulloch[27] established the spleen colony assay to define the mouse HSC with its ability to self-renew. Since then, the potential for differentiation and self-renewal has been considered to be two classical fundamental properties of a stem cell. However, these two properties must be retrospectively demonstrated, which is never an easy task in research.

The field of stem cell research has been significantly shaped by the isolation of mouse HSCs using flow cytometry, a process that can identify a definitive immunophenotype.[28] However, the specific immunophenotype of a stem cell is not fixed and it could be a moving target depending on many factors such as age, strain, species and host conditioning[29,30] (H Shen and T Cheng, unpublished data). Even for the 'best defined' mouse HSC, there is no single immunophenotype that has been used by everyone in the field. There is currently an emphasis on functional approaches to stem cell identification such as Hoechst efflux;[31–34] however, these approaches have not been able to offer a definitive solution.[35] In general, functional and phenotypic characteristics are both important in defining a tissue stem cell, but the two are not always associated with each other. Unfortunately, stem cell rarity in the body precludes us from identifying stem cell types by the use of many conventional molecular analyses. Therefore, unlike many other tissue cell types, the definition of stem cell types at cellular and molecular levels is often nonoptimal and could be considered arbitrary in many studies. The variability that exists within what is defined as a tissue stem cell type of any particular organ may be largely responsible for discrepancies between published data regarding specific stem cell types. Therefore, one of the most important subjects for stem cell research is the continuing effort to define stem cell identity.

Embryonic stem cells versus adult stem cells

Perhaps the greatest cause of confusion for the general public—and even in some researchers' minds—is the use of the term 'stem cells' to describe both ESCs and tissue or adult stem cells (ASCs), two major general stem cell types. (For the purposes of this paper, I will use ASCs to represent all somatic tissue stem cells except ESCs, and therefore ASCs do not necessarily refer to stem cells from adults.) Although these stem cell types differ in many fundamental ways, they are often regarded as a similar cell type. This misconception not only causes biological inaccuracies in describing these cells but also generates problems in the ethical debates over stem cell research. Given this situation, I would like to reiterate the key differences between ESCs and ASCs as illustrated in Table 1. In short, ESCs are cultured cell lines and an ESC equivalent has yet to be discovered *in vivo*. In contrast, ASCs are more physiologically relevant and thus can be readily used for autologous transplant and if a specific immune barrier can be managed—in the setting of allotransplant.

Table 1 Fundamental differences between ESC and ASC

	ESC	*ASC*
Origin	Blastocyst	Developed tissues
Proliferation *in vitro*	Indefinite	Limited
Differentiation spectrum	All the tissue types	Limited
Homing ability	No	Yes
Reconstitution efficiency	Low	High
Tumorigenesis	Teratoma	No or rare
Availability	Restricted (human)	Less restricted
Ethical issues	Severe (human subjects or human-xeno models)	Less
Clinical proof	No	Yes (HSC)
The most challenging technique in therapeutics	Efficient induction of specific tissue types without tumorigenesis	*In vitro* expansion without loss of physiological properties

Abbreviations: ASC, adult stem cell; ESC, embryonic stem cell; HSC, hematopoietic stem cell.

It should be emphasized that the comparison in Table 1 does not suggest a superior importance of one stem cell type over another. Generally speaking, both are equally important. The choice of ESCs versus ASCs in therapeutics is dependent upon the specific type of diseased tissue that is receiving therapy. For some tissues, such as the pancreas in which ASCs have not been shown to be essential in tissue regeneration,[36] ESCs may be more valuable as a source of making functional β cells. In contrast, HSCs are known to be fully responsible for regenerating all of the blood cell lineages. If the current hurdle of HSC expansion can be overcome, we may not have to use ESC-derived HSCs for HSC therapy in some clinical circumstances such as autologous HSC transplant.

In addition to the individual uses to which ESCs and ASCs can be put, research into each of these types is critical to understanding the other. ESCs can be precursor cells for ASCs, and this process offers a useful model to study how ASCs are generated. As a result, the 'tricks' for expanding ASCs may be uncovered by studying ESC biology. On the other hand, understanding how ASCs operate physiologically is pivotal for the development of strategies for coaxing ESCs toward differentiation into therapeutically acceptable cells.

Critical aspects on the physiology of stem cells

Our ability to manipulate stem cells for therapeutic purposes is highly dependent on our understanding of

the mechanisms that underlie stem cell kinetics *in vivo*. Stem cells are defined by their capacity for self-renewal and differentiation, but there are only two multiple cellular functions that are critical for maintaining the homeostasis of ASCs *in vivo*.

Self-renewal

It is a common misconception that all stem cell self-renewal occurs in the same way that general cells proliferate. In fact, stem cells show two different methods of self-renewing: one is asymmetrical (one daughter stem cell and one differentiated cell after division) and the other is symmetrical (two daughter stem cells after division). ESCs can only undergo symmetrical self-renewing division, whereas ASCs (for example, HSCs) and neural stem cells (NSCs) are thought to undergo asymmetrical self-renewing division under homeostatic conditions.[37] At the cell population level, stem cells self-replicate at a probability between 0 and 1.0.[20] Thus, stem cell maintenance requires probability of 0.5 and a probability of less or more than 0.5 can result in exhaustion or expansion, respectively. The acceleration of HSC proliferation often results in the loss of HSC self-renewal potential after birth.[38–40] The molecular basis for this cellular phenomenon has been shown in mice deficient in several cell cycle regulators such as p21 (Cip1/Waf1), Gfi-1 and Pten.[39–41]

Because ESCs and ASCs self-renew in different manners, it is unlikely that the same molecular circuitry governs the self-renewal process in both cell types. It has been shown that transcription programs dictated by Oct4, Nanog and Sox2 play essential roles in ESC self-renewal.[42] In ASCs, the mechanisms appear to be much more complex, although Bmi-1 has been shown to be shared between some ASC types, including HSCs, NSCs and mammary stem cells.[43–46] Interestingly, this mechanism is also shared by some TSCs.[44] As a cell cycle inhibitor, p18 has been shown to play a unique role in the self-renewal of several stem cell types, including HSCs, lung stem cells and possibly NSCs.[47,48] While self-renewal enables stem cells to continue even beyond the lifetime of the organisms from which they are harvested,[49] this ability appears to decrease with increasing age of the cell.[50] Ataxia telangiectasia mutated and p16 appear to play important roles in this decrease.[51–53]

Maturation (differentiation)

Differentiation is the most direct cellular basis for using stem cells in regenerative medicine. All stem cells are able to differentiate into specified cell types under specific conditions. The spectrum of differentiation depends upon the stem cell type and the microenvironmental cues it receives. Unlike ESCs, which can differentiate into virtually any kind of cell in the body, the plasticity of ASCs toward different tissue types appears to be limited,[54–56] although this issue continues to be debatable depending upon the identity of actual input 'stem cells' in a given study.[57,58]

The molecular basis for the choice between self-renewal and differentiation is the central question in stem cell biology. There have been two general theories regarding the mechanisms: instructive (environment-dependent) and stochastic (environment-independent).[19,59] As mentioned above, unlike ESCs in which differentiation and self-renewal are uncoupled, ASCs can undergo asymmetrical division resulting in concurrence of self-renewal and differentiation.[60,61] As largely demonstrated in hematopoiesis, differentiation is a sequential process that ultimately yields fully mature cell populations via hematopoietic progenitor cells,[62] although this was challenged by an alternative model in which a specific cell cycle position, rather than a hierarchical position in the differentiation cascade, determines whether a primitive cell functions as a stem or a progenitor cell.[63] According to the hierarchical model, however, stem cell number is not the sole determinant for the size of the final mature cells. In fact, because intermediate progenitor cells directly yield mature cell populations, self-regeneration of these progenitor cells must also be crucial or even more important in producing mature cells under homeostatic conditions.[25] Therefore, factors regulating stem cell repopulation efficiency (meaning the function per stem cell) are important but have been less emphasized, although this paradigm was partially illustrated in the absence of p27.[64]

Apoptosis (cell death)

In theory, apoptosis is also a fate choice of stem cells. But under homeostatic conditions, in which stem cells undergo asymmetrical divisions to maintain a constant number of stem cells, apoptosis may be a less frequent event. However, once symmetrical self-renewing division occurs and the stem cell pool size is increased, apoptosis is likely one of the mechanisms used to maintain the stem cell pool at its proper size. Stem cells must have the capability to die in order to prevent the overgrowth of stem cells that may lead to cancer.

In general, stem cell apoptosis is a relatively poorly studied area. Although the antiapoptotic protein Bcl-2 has been shown to increase HSC number in a transgenic model,[65] whether it acts directly via suppression of an apoptosis mechanism remains to be further defined, given the fact that Bcl-2 could also alter HSC cycling.[65] While the p53 pathway has been shown to be involved in stem cell senescence,[66,67] its direct role in stem cell apoptosis has not been clearly defined.

Resting mode (quiescence)

Adult stem cells have been thought to be resistant to many physiological stimuli and pathophysiological insults due to their ability to maintain themselves for the lifetime of an organism. It is known that HSCs do not respond to many hematopoietic growth factors that affect hematopoietic progenitor cell populations under physiological conditions.[68–70] As a result, HSCs are relatively quiescent in cell cycle. Based on this characteristic, suicide approaches have been used to spare stem cells from their progeny when progenitor-specific growth factors and the S-phase-specific toxin 5-FU were sequentially applied in culture.[71–73] Therefore, the resting mode in which the majority of ASCs are maintained under homeostasis *in vivo* appears to be an important self-protective mechanism of the cells. However, the molecular basis for this cellular defense mechanism is largely unclear. While a number of potential molecular players such as p21, Gfi-1, MEF/ELF4 and angiopoietin signaling have been reported,[39,40,46,74] a common unifying mechanism has yet to be defined. In addition, potential

links of stem cell quiescence to other important cellular defense mechanisms, such as multidrug resistance and DNA repair possessed by stem cells,[75–77] are important areas that need to be investigated.

Trafficking (migration)

The ability of stem cells to traffic or migrate is best described with HSCs. HSCs have an active motility.[78,79] They constantly move in and out of their bone marrow niche and even circulate in the blood under homeostatic conditions.[80,81] Similarly, physiological stem cells are able to travel to their niche in a specific organ. This ability appears to be gradually acquired during embryonic development[82] and is a prerequisite for stem cells to reach and reside in damaged tissues in therapeutic scenarios. Therefore, the success of an HSC transplant is largely dependent upon the homing efficiency of transplanted HSCs in the marrow. Specific manipulations of homing molecules may enhance HSC engraftment efficiency upon transplantation. Such manipulation was exemplified in a recent study which demonstrated that targeting CD26 can significantly enhance HSC therapy efficiency in both mouse and human hematopoietic systems.[83,84]

Together, these five minimal functional states of stem cells (self-renewal, maturation, apoptosis, resting mode and trafficking) constitute an interesting 'SMART' model for maintaining stem cell homeostasis *in vivo* (Figure 1). In fact, critical roles of the multicellular niche in stem cell maintenance further reinforce the physiological definition of ASCs *in vivo* as illustrated by these functional features.[85,86] The lack of any of these 'SMART' features would make stem cells much less physiological and particularly useless in therapeutics. Given these highly complex and controlled features *in vivo*, the great variability of stem cell phenotypes might be largely due to specific functional states of given stem cells along these different fate choices. According to this model (that is certainly suitable for some well-defined ASCs such as HSCs), ESCs are apparently a more homogeneous but defective stem cell population because they can only self-renew in a symmetrical manner. ESCs do not have the ability to travel purposefully or migrate to an injured site and exert minimal or uncoupled functions of other features (Table 1). Even in the scenario where a differentiated cell type can be reprogrammed to an ES-like cell type[87,88] or ES equivalents can be generated via epiblasts,[89] these stem cell lines will have the same shortcomings as ESCs.

Unfortunately, however, TSCs also have these 'SMART' features and, in fact, TSCs must be able to outcompete their normal counterpart when they become a dominant phenotype. Regarding therapeutic manipulations, especially those involving genetic approaches, a fundamental challenge is to produce therapeutically acceptable stem cell products while avoiding the production of tumorigenic stem cells. For example, while overexpression of HoxB4 has been shown to be very effective in HSC expansion and leads to no increase of leukemogenesis in mice,[90,91] a recent study reported that dogs transplanted with HoxB4-expanded HSCs were susceptible to leukemia development.[92] Therefore, defining the molecular boundary between normal ASC and TSC is vital for the safe expansion of ASCs as well as for the development of methods to selectively target TSCs for therapeutic purposes.[93]

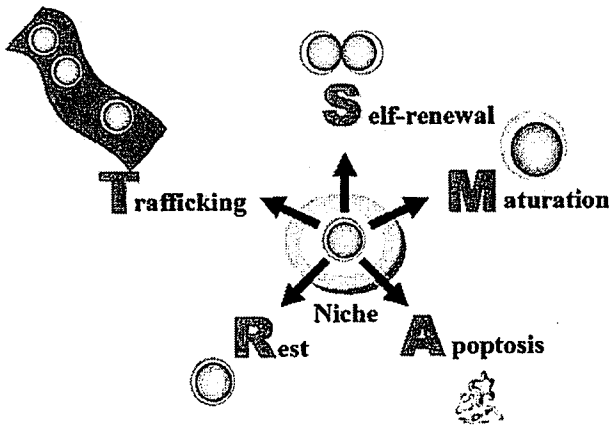

Figure 1 The 'SMART' physiological features of stem cells *in vivo*.

Final notes

Stem cell research shows promise for the treatment and/or cure of many devastating diseases for which we do not have effective treatments at the present time. Moreover, stem cell research provides new opportunities to increase the effectiveness of existing medical modalities. Our ability to manipulate stem cells for therapeutic purposes is directly dependent upon understanding the biology of this fascinating cell type. However, stem cell biology is still in its immature stage and thus has an enormous potential to grow. We are facing many critical challenges in its biological research as well as ethics. The 'SMART' model illustrated in this review (Figure 1) is not intended to offer a new definition for stem cells but rather to exemplify the physiological relevance of stem cells for their potential therapeutic applications. It should also be emphasized that stem cell therapy cannot stand by itself and combinational therapies, such as a combined stem cell and gene therapy, are likely the common scenarios for future clinical practice in this area.

In this special issue of *Gene Therapy*, which focuses on stem cells, limited space prevents us from covering all aspects of the field. Rather, we intend to showcase important topics that we feel have been relatively underemphasized in the literature. These topics are relevant but not limited to gene therapy, and all are directly or indirectly related to potential stem cell therapeutic development.

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